IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE MATTER OF: U.S. Patent Application Serial no. 09/866,569

Inventors: Bolton, Anthony R. et al

Filed: May 25, 2001;

Title: "Apoptotic Entities for Use in Treatment of Endothelium Dysfunction Disorders";

Group Art Unit: 1643;

Examiner: Yaen, Christopher H.;

Attorney docket: 355908-3100

DECLARATION

I, Dr. ARKADY MANDEL, M.D., Ph.D., D.Sc., medical doctor, hereby declare:

1. THAT I am currently Director of Fundamental and Medical Research at Vasogen Inc., Mississauga, Ontario, the parent company of the owner of the patent application identified above and I have held this position since October 9, 2000. I joined Vasogen Inc. on September 1, 1997 as a senior research scientist. I am also listed as a joint inventor on the instant patent application.

2. THAT I am a medical doctor and hold a Ph.D. and Doctor of Medical Science. I have authored or co-authored over 60 scientific papers in the field of medicine, immunology, and dermatology over the last 24 years.

3. THAT I have read the disclosure and claims of the patent application, the prior art cited

by the Examiner in the Office Action mailed March 24, 2006, as well as the Office Action itself.

4. DNFB-induced contact hypersensitivity (CHS) is a Th-1 cell-mediated inflammatory disorder known to involve inflammatory cytokines such as IL-1, TNF-α, and IFN-γ and the degree of inflammation associated with CHS has been shown to be decreased by anti-inflammatory cytokines such as IL-10 (Watanabe *et al.*, Contact Hypersensitivity: The Mechanism of Immune Responses and T Cell Balance, (2002) *J. Interferon and Cytokine Res.*, 22: 407-412; Nakae *et al.*, IL-1-induced Tumor Necrosis Factor-α Elicits Inflammatory Cell Infiltration in the Skin by Inducing IFN-γ-inducible Protein 10 in the Elicitation Phase of the Contact Hypersensitivity Response, (2003) *Int. Immuno.*, Vol. 15, No. 2, 251-260; Kondo *et al.*, Epidermal Cytokines in Allergic Contact Dermatitis, *J. Am. Acad. Dermat.*, (November 1995) Vol. 33, No. 5, 786-800).

5. Inflammatory cytokines are understood to play a role in the pathophysiology of congestive heart failure (CHF) (Parissis *et al.*, An Overview of Inflammatory Cytokine Cascade in Chronic Heart Failure, *Hellenic J. Cardiol.*, (2002) 43: 18-28). CHF patients have been shown to have persistent immune activation *in vivo* characterized by increased circulating levels of inflammatory cytokines such as IL-1β, TNF-α, and IL-6 as well as enhanced expression of inflammatory mediators such as TNF-α and IL-6 within the failing myocardium, independent of the cause of CHF (Damas *et al.*, Cytokines as New Treatment Targets in Chronic Heart Failure, (2001) *Curr. Control. Trials Cardiovasc. Med.*, 2: 271-277).

6. An initial study on a therapy directed against TNF-α in patients with moderate heart failure showed promising results, although two larger studies showed no difference between the treatment group and placebo insofar as the primary endpoint (death or hospitalization because of CHF) and the secondary endpoint (all cause mortality) were concerned (Anker and von Haehling, Inflammatory Mediators in Chronic Heart Failure: an Overview, *Heart*, (2004) 90: 464-470).

7. Notwithstanding the negative results of paragraph 6 above, pentoxifylline, a compound shown to inhibit inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ, has been demonstrated, in combination with other heart drugs, in a small trial to be associated with a significant improvement in symptoms and left ventricular ejection fraction in patients suffering from idiopathic dilated cardiomyopathy (Damas, *supra*). Contrarily, researchers have shown that a decrease in IL-10 (an anti-inflammatory cytokine), as well as a decrease in the IL-10-to-TNF-α ratio, correlated with depressed cardiac function in heart failure in rats (Kaur *et al.*, Significance of Changes in TNF-alpha and IL-10 Levels in the Progression of Heart Failure Subsequent to Myocardial Infarction, *Am. J. Physiol. Heart Circ.l Physiol.* (July 2006) 291(1): H106-13).

8. It is well known that IL-10 is able to downregulate pro-inflammatory cytokines such as IL-1β and TNF-α. Therapy in CHF patients using intravenous immunoglobulin has been shown to increase IL-10 with a slight decrease in TNF-α and IL-1β, suggesting that administration of anti-inflammatory cytokines such as IL-10 may be a useful strategy for

treatment of CHF patients (Damas, *supra*).

9. It is my opinion that the literature cited above, evidences that decreases in pro-inflammatory cytokines and/or increase in anti-inflammatory cytokines would correlate to treatment protocols for CHF. Based on the evidence in U.S. Serial no. 09/866,569, Examples 1 and 2 that show a decrease in inflammation in the CHS assay using the claimed methods, the skilled artisan, in view of the cited literature and the teachings of the present application, would correlate these results from the CHS model to treatment of CHF.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

EXECUTED at Mississauga, Ontario, Canada, this 21st day of August, 2006.

Arkady Mandel